Fibroblast cell-substratum interactions: Role of cold insoluble globulin (plasma fibronectin)

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The adhesion of cells to an extracellular matrix is a complex process involving both cell surface and cell cytoskeletal components. 3 central problems have emerged in trying to understand this phenomenon. These are: the nature of the interaction between the cells and the substratum; the mechanism by which the initial cell-substratum interaction triggers the subsequent reorganization of the cytoskeleton that causes cell spreading. A review of cellular adhesiveness and extracellular substrata has been published recently[2] which deals with adhesion in a comprehensive manner, not only in terms of the various problems mentioned above, but also, with respect to the various types of cells whose adhesive properties have been studied. The purpose of the present paper is to review several aspects of our current understanding about the nature of the cell-substratum interaction.

Specificity of cell-substratum interactions

Recent findings indicate that cell adhesion can be a general response of cells to specific ligand-receptor interactions between the cell surface and the substratum. There does not appear to be a requirement for a unique cell surface receptor or substratum site. Rather, a variety of ligands known to bind to different cell surface components are able to support cell adhesion when the ligands are first adsorbed to an underlying material surface (e.g., plastic, glass, etc.). Examples are: antibodies against cell surface antigens[3,4], plant lectins[3,5-7], polycationic proteins[3,8-10], and other proteins for which there are specific cell surface receptors[11-13] (e.g., adsorbed asialoceruloplasm induces adhesion of hepatocytes[13]). These findings suggest that in situ cells potentially can undergo a variety of different adhesive interactions in response to the chemical make-up of different matrices. The only requirement may be that ligand-receptor interactions occur between the matrix and the cell surface.

It should be pointed out that although a variety of ligand-receptor interactions promote cell adhesion, the mechanism of the cytoskeletal triggering response may be the same in every case. At least, this was the conclusion of a study in which several kinds of ligand induced cell spreading responses were compared[3]. This situation would be analogous to the capping phenomenon in which a variety of ligands induce capping of different cell surface receptors[14], probably through a common cytoskeletal triggering-response mechanism[15,16].

Cold insoluble globulin dependence of cell adhesion to material surfaces

Although cell adhesion may be induced by a variety of ligands directed against the cell surface, 1 particular factor found in plasma and serum has been implicated in the adhesion of fibroblasts in vitro and may play a major role in the adhesion of these cells in situ. This is the ubiquitous, well-characterized glycoprotein, cold insoluble globulin (CIG)[17,18] (plasma fibronectin). The dependence of cell adhesion on a serum protein was reported more than 20 years ago when fetuin was shown to be a cell adhesion factor[19]. The properties of the active component, which was shown to be a glycoprotein contaminant of fetuin, indicate that it was probably CIG[8,20]. A systematic analysis of the serum factors involved in fibroblast adhesion was again initiated several years ago utilizing a cell spreading assay[21,22]. The factor was isolated from fetal calf serum and biochemically characterized. There appeared to be several active components, one of which was similar to CIG[23]. Subsequent experiments using human serum confirmed that CIG was the factor involved[24].

It was shown that CIG must be adsorbed to a material surface in order for activity to be observed[22]. Adding CIG to the incubation medium did not promote adhesion if some other protein (albumin) was adsorbed to the material surface. Moreover, the density of CIG molecules adsorbed on the material surface controlled the extent of cell attachment as well as the extent of cell spreading. With low concentrations of adsorbed CIG, attachment occurred but not spreading[23]. Recent findings indicate that about 45,000 CIG molecules must be present beneath the cells to promote complete cell spreading[7].

The reactive groups of CIG that are important in promoting cell adhesion were analyzed by determining the effects of chemical modifications on the activity of CIG after it was adsorbed to the material surface. It was found that treatments known to block carboxyl groups, tyrosine residues or tryptophan residues inhibited activity. Treatments known to block amino groups, sulfhydryl groups, or carbohydrate portions of the molecule were without effect[25].

CIG dependence of cell adhesion to collagen

A serum protein was also shown to be involved in cell adhesion to collagen[26] and subsequently it became apparent that CIG was the protein involved[27]. The site on collagen to which CIG binds[28] and fragments

of the CIG molecule which contain the collagen binding site[29-31] have been isolated.

The early studies on CIG interaction with collagen were carried out with substrata composed of denatured collagen gels and CIG was found to be absolutely required for adhesion to these substrata[26]. However, in studies comparing several different native and denatured collagen substrata, it was observed that CIG was not absolutely required for adhesion to native collagen[32]. Others have also observed CIG independent adhesion to native collagen, however, CIG promotes the interaction[33,34]. The direct interaction of fibroblasts with collagen is not surprising since these cells appear to have a collagen receptor on the cell surface[35].

CIG dependence of cell adhesion to fibrin

Recent studies on fibroblast adhesion to fibrin and fibrinogen have shown that fibroblasts are not able to attach directly to material surfaces coated by either of these 2 proteins[36]. Addition of CIG was found to promote the adhesion of fibroblasts to fibrinogen or fibrin, and much lower concentrations of CIG were effective if the CIG was first incubated with fibrinogen or fibrin substrata under conditions whereby the CIG became covalently linked through the action of factor XIII. The ability of factor XIII to crosslink CIG to fibrin was reported previously[37].

Adhesion of diploid fibroblasts

Most of the studies described above were carried out with permanent cell lines (e.g., baby hamster kidney cells, Chinese hamster ovary cells). On the other hand, diploid fibroblasts behave somewhat differently in that such cells attach and spread on material surfaces or denatured collagen without the addition of CIG[32,38-40]. It is known that CIG is closely related to the fibroblast cell surface protein called fibronectin[41] (see Vaheri and Mosher[42] and Yamada and Olden[43] for recent reviews). Since diploid cells generally have higher levels of fibronectin and secrete more of this material into the medium than permanent cell lines[44-46], it seemed likely that such cells might secrete their own fibronectin onto the material surface and initially interact with the secreted fibronectin[2]. Recent studies have confirmed this hypothesis[47]. That is, early passage human skin fibroblasts were found to secrete fibronectin locally onto the material surface and then interact with the fibronectin. It was also found that cells harvested from post-confluent cultures, in which a large extracellular fibronectin matrix can be observed, did not secrete sufficient fibronectin to attach and spread and required the addition of exogenous CIG. This suggests that fibronectin synthesis is shut down once cells are in a suitable matrix; therefore, sessile fibroblasts in situ may not be secreting fibronectin.

Cell surface receptor for CIG

Finally, it is appropriate to comment on current studies attempting to identify the cell surface receptor which interacts with CIG. This problem has been a difficult one because soluble CIG does not bind strongly to cells, although binding can be detected by immunofluorescence. For instance, addition of excess CIG does not decrease the interaction of cells with CIG adsorbed to a material surface and pretreatment of cells in suspension with CIG does not render them subsequently able to attach and spread on an untreated material surface (unpublished observations). At the present time, it seems likely that soluble CIG interacts weakly with the cells, and that the strength of this interaction is dramatically (exponentially) increased when the CIG is first adsorbed on the material surface. Most likely, this is because of the cooperative effect of multiple binding interactions[2].

So far, in most of the work on identifying the receptor, indirect methods of analysis have been used. Based upon these studies, there is evidence suggesting the following as possible receptors: glycosaminoglycans[48-50] (especially heparan sulfate), glycolipids[51] (especially GDla and GTI), the ricin receptor[52], and actin stress fibres[53,54]. Recently, we have developed a direct method for analyzing the CIG receptor[55]. Polystyrene latex beads (0.8 μm) that do not ordinarily interact with baby hamster kidney fibroblasts have been coated with CIG. When incubated with cells at 4 °C, the CIG beads were observed to bind all over the surfaces of cells in suspension. At 37 °C binding of CIG beads was followed by endocytosis and cell aggregation. Pretreatment of cells with 0.1 mg/ml of trypsin for 10 min at 37 °C inhibited subsequent binding. On the other hand, EDTA and EGTA did not inhibit binding. Pretreatment of cells with the lectin, wheat germ agglutinin, also inhibited subsequent binding of CIG beads. These preliminary findings suggest that the CIG receptor is a protein and carbohydrate-containing component, uniformly distributed on the cell surface, that it is active without undergoing reorganization in the membrane, and does not require divalent cations for stability or activity.

Conclusions

The evidence indicates that cell-substratum interactions are mediated by specific ligand-receptor interactions between appropriate cell surface receptors and substratum sites. For fibroblasts, the major substratum site of adhesion appears to be CIG, or fibronectin in the case of those fibroblasts that secrete their own

adhesion factor. The fact that these glycoproteins are involved in adhesion to collagen and fibrin as well as material surfaces indicates that they probably play a major role in fibroblast adhesion in situ.

Current evidence indicates that CIG and fibronectin exhibit somewhat distinct biological activities in in vitro assays for adhesion[56] despite their immunological cross-reactivity and many other similar physical properties[57,58]. The CIG/fibronectin antigen is present predominantly in the connective tissue and basement membranes of adult tissues[59,60]; however, the extent to which extravascular CIG contributes to this distribution has not yet been established. In addition, the source of CIG is unknown. The possibility that fibronectin that has been secreted by fibroblasts[61] or endothelial cells[62–64] is processed to form CIG is an attractive hypothesis lacking direct evidence. Therefore, the precise relationship between CIG and fibronectin and the possibility that these molecules play different roles in adhesion in situ are unresolved problems.

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